

Amendments to the Claims:

Please amend the claims as set forth hereinafter.

Listing of Claims:

This listing of claims will replace all prior versions, and listings, of claims in the application:

1. to 47. (Cancelled)

48. (Currently Amended) A method for detecting an interaction between a first membrane bound protein or part thereof and a second protein or part thereof, which is either membrane bound or soluble, the method comprising:

(a) providing a host cell containing at least one detectable gene (reporter gene) having a binding site for a transcriptional activator, such that the detectable gene expresses a detectable product when the detectable gene is transcriptionally activated;

(b) providing, as part of a bait vector, a first chimeric gene under the control of a promoter, said first chimeric gene being expressed in said host cell and coding *inter alia* for a first membrane protein or part thereof which gene is attached to the DNA-sequence of a first module encoding *inter alia* a first protein sequence involved in intracellular protein degradation and a transcriptional activator, said first protein or part thereof to be tested whether it can interact with a second protein or part thereof, wherein said bait vector is a plasmid construct;

(c) providing, as a part of a prey vector, a second chimeric gene under the control of a promoter that can be expressed in said host cell, the second chimeric gene coding *inter alia* for a second protein or part thereof which is either membrane bound or soluble and which gene is attached to the DNA sequence of a second module encoding *inter alia* a second protein sequence involved in intracellular protein degradation, wherein said prey vector is a plasmid construct;

(d) introducing the bait vector and the prey vector into the host cell such that an interaction between the expressed first and second proteins and/or their parts can take place, which interaction leads to an interaction of the first protein sequence of the first module and the second protein sequence of the second module which

interaction in turn leads to activation of an intracellular protease and proteolytic separation of the transcriptional activator, wherein both the bait vector and the prey vector are maintained episomally;

(e) determining whether the detectable gene of the host cell has been activated by the transcriptional activator; and

(f) detecting said interaction between said first membrane bound protein or part thereof and said second protein or part thereof.

49. (Previously Presented) The method according to claim 48, wherein the host cell is a yeast, a bacterium or a mammalian cell.

50. (Previously Presented) The method according to claim 49, wherein the yeast is *Saccharomyces pombe* or *Saccharomyces cerevisiae*.

51. (Previously Presented) The method according to claim 48, wherein the detectable gene is activated by an activator comprising a short tagging module.

52. (Previously Presented) The method according to claim 48, wherein the detectable gene is activated by the artificial transcriptional activator protein A-LexA-V16 (PLV).

53. (Previously Presented) The method according to claim 48, wherein the first protein sequence comprises a C-terminal portion of ubiquitin (Cub) or a mutant thereof (CbM) and the second protein sequence comprises an N-terminal portion of ubiquitin (Nub) or a mutant thereof (NbM).

54. (Previously Presented) The method according to claim 48, wherein the DNA-sequence coding for the first membrane protein is selected from the group consisting of any bacterial membrane protein, any viral membrane protein, any oncogene-encoded membrane protein, any growth factor receptor or any eukaryotic membrane protein, or parts thereof.

55. (Previously Presented) The method according to claim 48, wherein the second membrane protein or the soluble protein, or part thereof, is encoded by a plasmid

library.

56. (Currently Amended) The method according to claim 48, wherein the first membrane protein is a soluble protein attached artificially to the membrane via fusion to a signal sequence which encodes a membrane anchor.

57. (Currently Amended) A kit for detecting binding between a first membrane bound protein or part thereof and a second protein or part thereof which is either membrane bound or soluble comprising:

- (a) a host cell containing at least one detectable gene (reporter gene) having a binding site for a transcriptional activator, such that the detectable gene expresses a detectable product when the detectable gene is transcriptionally activated;
- (b) a first vector (bait), which is a plamid construct that is maintained episomally, comprising a first site that can receive a first nucleic acid coding for a first membrane protein or part thereof such that when the first nucleic acid is inserted it becomes attached to the DNA sequence of a first module encoding *inter alia* a first protein sequence involved in intracellular protein degradation, the first module further comprising a nucleic acid for a transcriptional activator and a promoter;
- (c) a second vector (prey), which is a plamid construct that is maintained episomally, comprising a second site that can receive a second nucleic acid coding for a second membrane protein or a soluble protein or part thereof such that when the second nucleic acid is inserted it becomes attached to the DNA sequence of a second module encoding *inter alia* a sequence protein sequence involved in intracellular protein degradation, wherein the second module further comprises a promoter; and optionally
- (d) a plasmid library encoding second proteins or parts thereof, wherein binding between said first membrane bound protein or part thereof and said second protein or part thereof is detected.

58. (Previously Presented) The kit according to claim 57, wherein the host cell is a yeast, a bacterium or a mammalian cell.

59. (Previously Presented) The kit according to claim 58, wherein the yeast is *Saccharomyces pombe* or *Saccharomyces cerevisiae*.

60. (Previously Presented) The kit according to claim 57, wherein the detectable gene can be activated by an activator comprising a short tagging module.

61. (Previously Presented) The kit according to claim 57, wherein the detectable gene can be activated by the artificial transcriptional activator protein A-LexA-V16 (PLV).

62. (Previously Presented) The kit according to claim 57, wherein the first protein sequence contains Cub or CbM and the second protein sequence contains Nub or NbM.

63. (Previously Presented) The kit according to claim 57, wherein the promoter in (b) is a CYC1 promoter or a CUP1 promoter.

64. (Previously Presented) The kit according to claim 57, wherein the DNA sequence coding for the first membrane protein is derived from any bacterial membrane protein, any viral membrane protein, any oncogene-encoded membrane protein, any growth factor receptor or any eukaryotic membrane protein, or parts thereof.

65. (Previously Presented) The kit according to claim 57, wherein the DNA sequence coding for the second protein is contained in a plasmid library.

66. (Currently Amended) A vector comprising method for detecting an interaction between a first membrane bound protein or part thereof and a second protein or part thereof, which is either membrane bound or soluble, the method comprising:  
(a) providing a host cell containing at least one detectable gene (reporter gene) having a binding site for a transcriptional activator, such that the detectable gene expresses a detectable product when the detectable gene is transcriptionally activated;  
(b) providing, as part of a bait vector, a first chimeric gene under the control of a promoter, said first chimeric gene being expressed in said host cell and coding *inter alia* for a first membrane protein or part thereof which gene is attached to the

DNA-sequence of a first module encoding *inter alia* a first protein sequence involved in intracellular protein degradation and a transcriptional activator, said first protein or part thereof to be tested whether it can interact with a second protein or part thereof, wherein the bait vector comprises the following elements:

- (a) a selection marker for propagation of the vector in *E. coli*;
- (b) an origin of replication which allows propagation of the vector in *E. coli*;
- (c) a further selection marker for propagation of the vector in yeast;
- (d) an origin of replication which allows episomal propagation of the vector in yeast; and
- (e) an expression cassette comprising the following elements:
  - (i) a promoter element;
  - (ii) a nucleic acid sequence encoding a leader selected from a signal sequence derived from a yeast integral membrane protein and a signal sequence, which confers fatty acid modification;
  - (iii) a nucleic acid sequence encoding Cub or CbM;
  - (iv) a nucleic acid sequence encoding a DNA binding protein; and
  - (v) a nucleic acid sequence encoding a transcriptional activator, wherein said vector is a plasmid;

(e) providing, as a part of a prey vector, a second chimeric gene under the control of a promoter that can be expressed in said host cell, the second chimeric gene encoding *inter alia* for a second protein or part thereof which is either membrane bound or soluble and which gene is attached to the DNA-sequence of a second module encoding *inter alia* a second protein sequence involved in intracellular protein degradation;

(d) introducing the bait vector and the prey vector into the host cell such that an interaction between the expressed first and second proteins and/or their parts can take place, which interaction leads to an interaction of the first protein sequence of the first module and the second protein sequence of the second module which interaction in turn leads to activation of an intracellular protease and proteolytic separation of the transcriptional activator, wherein both the bait vector and the prey vector are maintained episomally;

(e) determining whether the detectable gene of the host cell has been activated by the transcriptional activator; and

(f) detecting said interaction between said first membrane bound protein or part

thereof and said second protein or part thereof.

67. (Currently Amended) ~~The method of claim 66 comprising a~~ A host cell containing the bait vector of claim 66.

68. (Currently Amended) A method of identifying compounds, ~~which method comprises using~~ comprising providing the kit of claim 57 to and screening screen compounds for their ability to interfere with protein-protein interaction.

69. (Withdrawn – Currently Amended) A method for providing a compound that can interfere with protein/protein interaction, which method comprises:

- (a) providing a host cell according to claim 67, the bait and prey polypeptides being selected such that they interact when expressed;
- (b) incubating the host cell in the presence and absence of the compound(s) to be tested;
- (c) measuring the difference in reporter gene expression between the incubation containing the compound(s) to be tested and the incubation free of the compound(s) to be tested; and optionally
- (d) purifying or synthesizing the compound that ~~can~~ interferes with protein-protein interaction.

70. (Previously Presented) The method of claim 68, wherein said compound is a pharmaceutical drug.

71. (Previously Presented) The method of claim 48, wherein the bait vector is a low copy vector.

72. (Previously Presented) The method of claim 71, wherein the bait vector is present in 1 to 2 copies per cell.

73. (Currently Amended) ~~The method~~ vector of claim 66, wherein said origin of replication of ~~the bait vector~~ in (d) is a CEN/ARS origin of replication.

74. (Currently Amended) ~~The method~~ vector of claim 73, wherein the signal

sequence of the bait vector encoded in (e)(ii) ~~(b)(iii)~~ is: N-MGCTLSAEDKPGGP-C (SEQ ID No. 1).

75. (Currently Amended) A vector of claim 48, wherein method for detecting an interaction between a first membrane bound protein or part thereof and a second protein or part thereof, which is either membrane bound or soluble, the method comprising:

(a) providing a host cell containing at least one detectable gene (reporter gene) having a binding site for a transcriptional activator, such that the detectable gene expresses a detectable product when the detectable gene is transcriptionally activated;

(b) providing, as part of a bait vector, a first chimeric gene under the control of a promoter, said first chimeric gene being expressed in said host cell and coding *inter alia* for a first membrane protein or part thereof which gene is attached to the DNA sequence of a first module encoding *inter alia* a first protein sequence involved in intracellular protein degradation and a transcriptional activator, said first protein or part thereof to be tested whether it can interact with a second protein or part thereof and said promoter in (b) is being a promoter that confers low level expression;

(c) providing, as a part of a prey vector, a second chimeric gene under the control of a promoter that can be expressed in said host cell, the second chimeric gene coding *inter alia* for a second protein or part thereof which is either membrane bound or soluble and which gene is attached to the DNA sequence of a second module encoding *inter alia* a second protein sequence involved in intracellular protein degradation;

(d) introducing the bait vector and the prey vector into the host cell such that an interaction between the expressed first and second proteins and/or their parts can take place, which interaction leads to an interaction of the first protein sequence of the first module and the second protein sequence of the second module which interaction in turn leads to activation of an intracellular protease and proteolytic separation of the transcriptional activator, wherein both the bait vector and the prey vector are maintained episomally;

(e) determining whether the detectable gene of the host cell has been activated by the transcriptional activator; and

~~(f) detecting said interaction between said first membrane bound protein or part thereof and said second protein or part thereof.~~

76. (Currently Amended) The ~~method~~ vector of claim 75, wherein said promoter in (b) is a CYC1 promoter ~~or a CUP1 promoter~~.

77. (New) The vector of claim 75, wherein said promoter in (b) is a CUP1 promoter.

78. (New) The method of claim 48, wherein the bait vector propagates via a CEN/ ARS origin of replication.

79. (New) The method of claim 78, wherein the prey vector propagates via a CEN/ ARS or 2micron origin of replication.

80. (New) The kit of claim 57, wherein the first vector propagates via a CEN/ARS origin of replication.

81. (New) The kit of claim 80, wherein the second vector propagates via a CEN/ARS or 2micron origin of replication.